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Passive Transfer of Respiratory Syncytial Virus (RSV) Antiserum Suppresses the Immune Response to the RSV Fusion (F) and Large (G) Glycoproteins Expressed by Recombinant Vaccinia Viruses

BRIAN R. MURPHY,[1]* ROBERT A. OLMSTED,[1] PETER L. COLLINS,[1]
ROBERT M. CHANOCK,[1] AND GREGORY A. PRINCE[2]

Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892,[1] and Department of International Health, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland 21205[2]

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In young infants who possess maternally derived respiratory syncytial virus (RSV) antibodies, the antibody response to RSV glycoproteins is relatively poor, despite extensive replication of RSV. In the present study, it was found that cotton rat RSV hyperimmune antiserum suppressed the antibody response to the RSV glycoproteins but not the response to vaccinia virus antigens when the antiserum was passively transferred to cotton rats prior to infection with vaccinia recombinant viruses expressing the RSV envelope glycoproteins. The cotton rats which had their immune responses suppressed by passively transferred antibodies were more susceptible to infection with RSV than were animals inoculated with control serum lacking RSV antibodies. Furthermore, many of the immunosuppressed animals infected with the vaccinia recombinant viruses developed RSV glycoprotein antibodies which had abnormally low neutralizing activities. Thus, preexisting serum RSV antibodies had dramatic quantitative and qualitative effects on the immune response to RSV glycoproteins, which may explain, in part, the poor RSV antibody response of young human infants to infection with RSV. Our observations also suggest that immunosuppression by preexisting, passively acquired RSV antibodies may constitute a major obstacle to RSV immunoprophylaxis during early infancy, when immunization is most needed.

Respiratory syncytial virus (RSV) infection causes serious lower respiratory tract disease in infants and children and is unique among respiratory viruses in that the incidence of serious disease is highest among 2-month-old children (1). For this reason, immunization strategies to prevent RSV disease will involve immunization of very young infants. Recent studies of the immune response of infants and children undergoing primary RSV infection demonstrated that infants of less than 9 months of age produce much less antibody to both the fusion (F) and large (G) glycoproteins than do older infected infants and children (3). Further analysis of the factors that affect the poor immune response of the young infants revealed that both immunological immaturity and immunosuppression mediated by maternally derived antibodies played roles (2). The effect of maternally transferred RSV antibodies on the antibody response to the G glycoprotein was seen in infants as late as 6 to 8 months of age (2).

In the present study, we examined the effect of passively transferred RSV immune serum on the immune response induced by the F and G glycoproteins expressed by vaccinia virus-RSV recombinants. This system was chosen for two reasons. (i) The RSV immune serum should not affect the replication of the vaccinia virus vector; thus the levels of expression of RSV F and G glycoproteins in control groups should be comparable to those in animals receiving immune serum. (ii) Since vaccinia virus recombinants bearing glycoprotein genes of paramyxoviruses are being considered as live-virus vaccines for use in humans, their ability to stimulate an immune response in the presence of passively transferred antibodies needs to be evaluated quantitatively (5, 7, 9). We observed in this study that there is a dose-dependent suppression of the antibody response to the vaccinia virus-expressed RSV F and G glycoproteins by passively transferred RSV immune serum and that the immunosuppressed animals are more susceptible to RSV infection than nonsuppressed animals.

The design of this experiment involved the following steps: (i) passive transfer of 4 ml of RSV hyperimmune antiserum or normal cotton rat serum by intraperitoneal inoculation of 3- to 4-week-old cotton rats (*Sigmodon hispidus*, each weighing approximately 40 g) on day −1, (ii) inoculation of $10^{7.0}$ PFU of vaccinia virus recombinant intradermally (0.1 ml) on day 0, (iii) collection of blood from animals on days 0, 28, 56, and 91 for quantitation of the RSV F and G antibodies by using an enzyme-linked immunosorbent assay (ELISA) and a plaque reduction neutralization assay, (iv) challenge of animals intranasally on day 91 with $10^{5.5}$ PFU of RSV A2 (0.1 ml), and (v) harvest of lung and nasal turbinates on day 95 (i.e., on day 4 of RSV infection) for quantitation of virus replication. The methods for determining the titer of RSV in lung and nasal turbinates, for measuring neutralizing antibodies (60% plaque reduction), and for determining ELISA titers for antibody against F and G glycoproteins have been described elsewhere (6). The ELISA for vaccinia virus antibodies was performed as described previously (3), except that the antigen was purified vaccinia virus strain WR, used at a concentration of 266 μg/ml. The cotton rat hyperimmune antiserum was derived from cotton rats that had been infected intranasally and rechallenged twice with $10^{5.5}$ 50% tissue culture infective doses of RSV A2. This antiserum had a 60% plaque reduction titer of approximately 1:2,000.

On day 0, the levels of RSV F and G antibodies in serum

* Corresponding author.

BROADSIDE FIGURE CAPTIONS

TABLE 1. Effect of passive transfer of RSV immune serum on antibody response of cotton rats infected with vaccinia virus-RSV recombinants

Group (no. of rats)[a]	Serum and dilution[b]	Serum ELISA titer (reciprocal mean $\log_2$ ± SE)[c] to:											
		RSV F glycoprotein				RSV G glycoprotein				Vaccinia virus			
		Day 0	Day 28	Day 56	% with response	Day 0	Day 28	Day 56	% with response	Day 0	Day 28	Day 56	% with response
	RSV												
A (9)	Undiluted	13.0 ± 0.25	9.8 ± 0.50	8.0 ± 0.83	33	14.5 ± 0.36	9.8 ± 0.33	7.5 ± 0.79	33	5.8 ± 0.62	18.2 ± 0.38	17.9 ± 0.39	100
B (5)	1:3	10.6 ± 1.80	10.7 ± 1.91	9.3 ± 1.88	60	12.0 ± 0.90	9.6 ± 0.79	9.3 ± 1.38	60	4.5 ± 0.70	17.3 ± 2.59	16.7 ± 2.44	100
C (7)	1:9	11.0 ± 0.28	11.0 ± 1.33	9.8 ± 1.42	57	10.7 ± 0.57	8.4 ± 0.59	9.5 ± 1.18	71	4.7 ± 0.57	15.5 ± 1.46	14.4 ± 1.13	100
D (6)	1:27	8.6 ± 1.36	11.9 ± 2.17	11.3 ± 2.05	82	8.9 ± 0.42	10.0 ± 1.33	10.4 ± 0.98	82	5.8 ± 1.21	16.7 ± 2.42	15.9 ± 2.35	100
E (7)	1:81	7.8 ± 0.37	15.5 ± 1.46	14.7 ± 0.57	100	7.0 ± 0.28	7.6 ± 1.59	8.7 ± 1.20	71	5.0 ± 0.52	16.0 ± 1.21	14.6 ± 1.03	100
F (9)	Undiluted	15.0 ± 0.25	11.0 ± 0.45	5.8 ± 0.62	NA	13.5 ± 0.25	9.5 ± 0.45	5.3 ± 0.75	NA	5.8 ± 0.33	19.2 ± 0.00	18.0 ± 0.40	100
G (8)	Control	3.3 ± 0.0	14.0 ± 0.52	12.5 ± 0.52	100	3.5 ± 0.25	13.7 ± 0.50	11.5 ± 0.29	100	3.8 ± 0.33	18.4 ± 0.36	17.2 ± 0.37	100

[a] Groups A through E and G received vaccinia virus-RSV recombinants expressing the F or G glycoproteins, and group F received a recombinant vaccinia virus expressing an unrelated antigen intradermally on day 0.
[b] Administered intraperitoneally on day −1. A 4-ml volume of control serum was used.
[c] An animal was considered to have a response if its antibody titer on day 56 was fourfold or greater than that of animals (group F) that received RSV immune serum and control vaccinia virus recombinant.
NA, Not applicable; unimmunized control group.

(Table 1) achieved in the passively immunized animals which received undiluted RSV antiserum were within the physiological range observed in infants during the first 2 months of life (4). The half-life of the passively transferred antibodies was approximately 6 days (Table 1, group F). By day 56, the titer of passively transferred RSV antibodies had diminished to almost background levels (Table 1, group F), allowing antibody responses to the vaccinia virus-expressed F and G glycoproteins to be detected unambiguously.

The presence of passively transferred RSV immune serum suppressed the antibody responses to the F and G glycoproteins in a dose-dependent manner (Table 1, groups A through E). Only 33% of the animals that received the largest amount of passive antibody (group A) developed an antibody response to the F or G glycoprotein, but each animal had a vigorous response to vaccinia virus antigens. The high titer of vaccinia virus antibodies that developed in each experimental group suggests that the passively administered RSV immune serum did not interfere with the replication of the vaccinia virus recombinants expressing the RSV glycoproteins. On day 56, the mean ELISA titers for antibody against the F and G glycoproteins of animals in group A were reduced 23- and 16-fold, respectively, compared with those in group G, which received normal serum and in which the response to vaccinia virus was not affected. This degree of suppression is equivalent to that observed in young infants

FIG. 1. Relationship between ELISA titer for F glycoprotein antibody and neutralizing-antibody titer. Preinfection (day 0) (△) and postinfection (day 56) (▲) sera were taken from eight animals which received normal control serum on day −1 and were infected intradermally with vaccinia virus-F glycoprotein and vaccinia virus-G glycoprotein recombinants on day 0. ——, Regression line defined by the pre- and postinfection sera of these eight animals ($r^2 = 0.94$); - - - - -, titers that are eightfold different from those described by the solid line. Circles indicate the titers of serum samples obtained on day 56 from 34 cotton rats which had been inoculated with RSV immune serum and then infected intradermally with vaccinia virus-F glycoprotein and vaccinia virus-G glycoprotein recombinants.

TABLE 2. Pattern of serum (day 56) RSV glycoprotein antibody responses of cotton rats receiving control or RSV immune serum prior to infection with vaccinia virus–RSV G- and F-glycoprotein recombinants

Serum and dilution	No. of rats tested	No. of animals with indicated pattern of antibody response (ELISA-F, ELISA-G, neutralization)[a]							
		H, H, H	H, L, H	L, H, H	L, L, H	H, H, L	H, L, L	L, H, L	L, L, L
Control (Undiluted)	8	8[b]	0	0	0	0	0	0	0
RSV immune									
1:81	7	3	0	0	0	2	2	0	0
1:27	6	3	0	0	0	2	0	0	1
1:9	7	2	0	0	0	1	1	2	1
1:3	5	2	0	1	0	0	1	0	1
Undiluted	9	0	0	1	0	1	2	1	4

[a] ELISA-F and ELISA-G, ELISA titers for F and G glycoprotein antibodies, respectively; H, high antibody titer, i.e., within fourfold of the mean of titers in the animals receiving control serum; L, low antibody titer.
[b] An additional 13 animals (previously studied) that received vaccinia virus–RSV G- and F-glycoprotein recombinants manifested this response pattern (5).

undergoing primary infection with RSV (2, 3). A suppressive effect on the response to the G glycoprotein was seen in group E even at the lowest dose of passive antibodies administered, which is consistent with the suppression of the G glycoprotein antibody response seen in older infants (2).

We then compared the ELISA titers for antibody against F glycoprotein with those for neutralizing antibody from immunosuppressed and control animals (Fig. 1; Table 1, groups A through E and group G, respectively). In animals that had received nonimmune control serum prior to immunization with the vaccinia virus recombinants, there was a positive correlation between the ELISA titers for F glycoprotein antibody and those for neutralizing antibody in serum samples collected on day 56, a result consistent with previous results (3). In contrast, a subset of 12 of 34 of the immunosuppressed animals developed a high ELISA titer for F glycoprotein antibody that was associated with low neutralizing activity. At any given ELISA titer for F glycoprotein antibody, the titer for neutralizing antibody in this subset showed a greater-than-eightfold reduction compared with that in animals which received control serum.

The patterns of antibody responses in control animals and in animals receiving passive RSV antibodies were further characterized by examination of the ELISA titers for F and G glycoprotein antibodies and the neutralizing-antibody response of individual animals (Table 2). Each of the eight control animals developed an immune response characterized by high ELISA titers for F and G glycoprotein antibodies and a high neutralizing-antibody titer. This has been a consistent finding in our other studies as well (5). Animals which had received the undiluted RSV immune serum occupied the other end of the spectrum, in that none developed a response with high ELISA titers for F and G glycoprotein antibodies associated with high neutralizing activity. Animals which received diluted RSV immune serum developed antibody patterns that remained intermediate between these two extremes. An important finding was the identification of animals that developed a high ELISA titer for F or G glycoprotein antibody that was not associated with appreciable neutralizing activity, which indicated that immunosuppression acted independently on the response to F and G glycoproteins. Furthermore, suppression was both qualitative and quantitative. In the latter instance, the total antibody responses to F and G glycoproteins were suppressed, while in the former instance, suppression was selective for epitopes involved in induction of neutralizing antibodies.

Immunosuppression effected by the passive RSV antibodies was associated with decreased resistance to RSV infection (Table 3). There was a dose-dependent effect of passive antibodies on the susceptibility to challenge with live RSV virus. Animals which received the highest dose of passive antibodies (group A) were the most susceptible to replication

TABLE 3. Immunosuppression of the F and G antibody responses by passive RSV antibodies increases susceptibility to RSV infection

Group (no. of rats)[a]	Serum and dilution	Neutralizing antibody titer (reciprocal mean $\log_2 \pm$ SE) on day 91	RSV replication in[b]:			
			Lungs		Nasal turbinates	
			% with virus recovered	Mean $\log_{10}$ virus titer (PFU/g $\pm$ SE)	% with virus recovered	Mean $\log_{10}$ virus titer (PFU/g $\pm$ SE)
F (9)	RSV Undiluted	3.4 ± 0.1	100	4.9 ± 0.3	100	5.1 ± 0.1
A (8)	Undiluted	4.0 ± 0.6	100	4.0 ± 0.4	100	4.9 ± 0.1
B (3)	1:3	5.3 ± 1.1	67	3.0 ± 1.1	88	4.8 ± 0.4
C (8)	1:9	7.2 ± 1.3	38	3.3 ± 0.6	100	4.4 ± 0.4
D (7)	1:27	4.4 ± 0.3	28	2.6 ± 0.5	100	4.3 ± 0.5
E (6)	1:81	6.7 ± 1.3	16	≦2.0 ± 0.0	50	3.7 ± 0.5
G (7)	Control	7.8 ± 0.3	29	2.1 ± 0.1	86	3.4 ± 0.3

[a] Vaccinia virus—RSV G- and F-glycoprotein recombinants were administered to groups A through E and group G. A vaccinia virus recombinant expressing an unrelated antigen was administered to group F.
[b] Animals were challenged with $10^{5.5}$ $TCID_{50}$ of RSV (0.1 ml) intranasally 91 days after receiving vaccinia virus recombinant, and their lungs and nasal turbinates were removed 4 days later for virus quantitation.

of the RSV challenge, and animals which received the lowest dose (group E) were the most resistant.

A major feature of RSV infection in humans is repeated reinfection during the first 5 years of life, with serious disease generally occurring during the first and second infections (1). The data from the present study demonstrate that passive RSV antibodies suppress the antibody response to RSV G and F glycoproteins expressed by recombinant vaccinia viruses and that these effects mimic the effects of maternally derived RSV antibodies on the immune response of young infants to infection with RSV. A subset of the immunosuppressed animals developed high titers of ELISA F- and G-glycoprotein-binding antibodies that had low neutralizing activity. In two of eight young infants (4 to 8 months of age) studied by us previously, an analogous elevated ratio of ELISA titer for F glycoprotein antibody to titer for neutralizing-antibody was seen in RSV convalescent-stage serum (2). The altered ratio of ELISA titer for F glycoprotein antibody to titer for neutralizing antibody seen in the cotton rats with preexisting RSV antibodies was also observed in a similar proportion of human infants possessing maternally derived antibodies at the time of RSV infection. This elevated ratio was not observed after infection of older infants and children but was seen in recipients of Formalin-inactivated RSV vaccine (4). Thus, the alteration in the immune response to RSV infection by passive RSV antibodies can take two forms: (i) an overall dose-dependent suppression and (ii) a qualitative alteration of the antibody response in which antibodies that possess functional activity (neutralizing) are disproportionately suppressed relative to ELISA-binding antibodies. It is likely that both forms of immune alteration play roles in the decreased resistance of the immunosuppressed animals to challenge with RSV, as well as in the susceptibility of infants and children to reinfection with RSV.

The phenomenon of antibody-mediated immunosuppression has been studied for several decades. The exact mechanism(s) by which passive antibody can suppress the immune response remains speculative; it is generally agreed, however, that precommitted B cells fail to expand in the immunosuppressed animal, but B-cell memory is stimulated and specific cellular immune responses are induced (8). Major goals in RSV research, therefore, will be to understand the cellular basis for antibody-mediated immunosuppression and to develop a strategy in which immunosuppression can be overcome, so that a protective immune response can be induced in very young infants who possess maternally derived RSV antibodies.

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